REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

(2)

AD-A241 560

2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	ANNUAL 01 Dec 90 TO 31 Nov 91

4. TITLE AND SUBTITLE
CELLULAR ANALOGS OF OPERANT BEHAVIOR

5. FUNDING NUMBERS
GR - AFOSR-89-0213
PE - 61102F
PR - 2312
TA - A1

6. AUTHOR(S)
Dr Larry Stein

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Department of Pharmacology
University of California
College of Medicine
Irvine, CA 92717

8. PERFORMING ORGANIZATION REPORT NUMBER
AFOSR-TR- 91 0761

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Dr Haddad
AFOSR/NL
Bu[illegible]g 410
Bol AFB DC 20332-6448

10. SPONSORING/MONITORING AGENCY REPORT NUMBER
91-13082

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

Our previous work indicates that hippocampal CA1 bursting may be reinforced by dopaminergic agents such as dopamine itself, cocaine, and certain dopamine receptor agonists. A major concern is that these agents may facilitate bursting merely by direct or indirect pharmacological stimulation of neuronal activity rather than by a cellular reinforcement process. We have always required as critical evidence of cellular reinforcement that noncontingent or random presentations of the positive agents will be relatively ineffective; and indeed random applications of dopamine, cocaine, and dynorphin A are ineffective and even tend to suppress the bursting of hippocampal pyramidal cells. One approach is to attempt to reinforce hippocampal bursting with a nonspecific depolarizing agent such as glutamate. Unlike dopamine and cocaine, burst-contingent applications of glutamate did not produce selective facilitation of cellular bursting when compared to ramdom presentations; indeed, both contingent and random glutamate applications reduced the likelihood of bursts, while at the same time increasing the frequency of individual spikes. These results are consistent with the idea that dopamine's reinforcing action on hippocampal bursting cannot be attributed to nonspecific stimulation. The burst-suppressant action of glutamate is intriguing, and suggests that glutamate mechanisms might normally function in opposition to the dopamine reinforcement mechanisms.

14. SUBJECT TERMS

15. NUMBER OF PAGES
4

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	UNLIMITED

AFOSR Grant 89-0213
Progress Report

Drs. Stein, McAfee & Belluzzi
31 Dec 1990

Good progress has been made in the Dec 89-Nov 90 period of our project "Cellular Analogs of Operant Behavior". The principal aims are to a) demonstrate the operant conditioning of individual cellular activity in an in vitro brain slice preparation and b) to determine whether or not such cellular operant conditioning underlies behavioral operant conditioning in vivo. If the two types of operant conditioning are interrelated, they should exhibit common properties. Thus, it might be possible to show that both forms of operant conditioning utilize the same reinforcing transmitters and drugs and that the same receptor subtypes mediate these reinforcing effects.

The work is organized in two main parts - cellular operant conditioning studies and behavioral operant conditioning studies.

I. Cellular Operant Conditioning

A. Opposite Effects of Dopamine and Glutamate on Hippocampal CA1 Operant Conditioning. (Xue, B.G. and Stein, L., *Soc. for Neurosci. Abstracts*, 16:261,1990.)

Our previous work indicates that hippocampal CA1 bursting may be reinforced by dopaminergic agents such as dopamine itself, cocaine, and certain dopamine receptor agonists. A major concern is that these agents may facilitate bursting merely by direct or indirect pharmacological stimulation of neuronal activity rather than by a cellular reinforcement process. We have always required as critical evidence of cellular reinforcement that noncontingent or random presentations of the positive agents will be relatively ineffective; and indeed random applications of dopamine, cocaine, and dynorphin A are ineffective and even tend to suppress the bursting of hippocampal pyramidal cells. One approach is to attempt to reinforce hippocampal bursting with a nonspecific depolarizing agent such as glutamate. Unlike dopamine and cocaine, burst-contingent applications of glutamate did not produce selective facilitation of cellular bursting when compared to random presentations; indeed, both contingent and random glutamate applications reduced the likelihood of bursts, while at the same time increasing the frequency of individual spikes. These results are consistent with the idea that dopamine's reinforcing action on hippocampal bursting cannot be attributed to nonspecific stimulation. The burst-suppressant action of glutamate is intriguing, and suggests that glutamate mechanisms might normally function in opposition to the dopamine reinforcement mechanisms.

B. Reinforcement of Hippocampal CA1 Bursting by Cannabinoid Receptor Activation. (Xue, B.G. and Stein, L., *Soc. for Neurosci. Abstracts*, in press.)

Involvement of cannabinoid receptors in behavioral reinforcement has been demonstrated in animals by self-administration of Δ^9-tetrahydrocannabinol (THC) and in humans by the addictive properties of marijuana and related agents. Furthermore, cannabinoid receptors and reinforcement-relevant dopamine D_2 and μ-opioid receptors are known to share the same signal transduction mechanisms and

have in common the ability to activate Gi proteins that inhibit adenylate cyclase. Accordingly, it was of particular interest to determine whether or not cellular operant conditioning could be demonstrated with cannabinoid receptor activation as reinforcement. The high affinity cannabinoid agonist CP-55940 was used as the reinforcer for CA1 hippocampal operant conditioning (cannabinoid receptors are present in high density in rat hippocampus). Highly reliable CA1 operant conditioning was obtained; more than 55% of the neurons tested were successfully reinforced by burst-contingent applications of CP-55940 (at concentrations of 5 and 10 μM, but not at 2.5 or 100 μM). The same microinjections, administered independently of firing, did not increase bursting rate and therefore provided a control for direct pharmacological stimulation of cellular activity. Co-administration of forskolin (which activates cyclic AMP formation) eliminated the reinforcing action of CP-55940, consistent with the idea that cannabinoid reinforcement may involve inhibition of cyclic AMP formation. The results indicate that cannabinoid receptor activation can reinforce hippocampal CA1 bursting and suggest that cannabinoid receptors, like dopamine and opioid receptors, may play important roles both in behavioral and cellular operant conditioning.

C. Dopamine Receptor Subtype and Cellular Reinforcement

Five dopamine receptors are presently recognized, which may be divided on the basis of homology and pharmacological similarity into two main dopamine receptor subgroups, D_1-like (D_1 and D_5) and D_2-like (D_2, D_3, D_4). In early experiments, we showed that the D_2-preferring agonist N-0437 was an effective reinforcer of hippocampal CA1 bursting activity, whereas the D_1 agonist SKF38393 was ineffective. To establish the specificity of N-0437's action at D_2 receptors, we compared the activity of its optical isomers, N-0923 and N-0924, which differ by 100-fold in D_2 potency. In the dose range 1-6 mM, only the D_2-active isomer N-0923 was effective as a reinforcer of CA1 bursting; even at the highest concentration of 6 mM, N-0924 was inactive. Most recently, we have been conducting CA1 operant conditioning using quinpirole, a D_3-preferring agonist, as reinforcement. Whereas the D_2 receptor is found in the majority of tissues innervated with dopamine, D_3 receptors are present in high densities only in motivationally-relevant limbic forebrain areas. To our surprise, quinpirole was active as a reinforcer at 0.025 mM--approximately 20 times more potent than dopamine itself. Quinpirole has 5 times greater affinity than dopamine for the D_3 receptor, and it also is more resistant to degradation--hence, the 20-fold potency differential is consistent with the possibility that the D_3 receptor subtype plays a major role in the mediation of reinforcement.

II. Behavioral Operant Conditioning

A. Mu-receptor mediation of dynorphin self-administration (Stevens, K.E., Shiotsu, G. and Stein, L., *Brain Res.*, 545:8-16, 1991.)

Drug-naive rats rapidly learned to self-administer dynorphin A in the CA3 region of hippocampus, confirming prediction from our earlier cellular work in which dynorphin A was found to reinforce the bursting activity of CA3 neurons. In subsequent behavioral studies, dynorphin A self-administration was blocked by co-administration of the opioid antagonist naloxone, which indicated that dynorphin's reinforcing effects are exerted at an opioid receptor. To establish which opioid receptor subtype may mediate dynorphin self-administration, co-administration studies were carried out with highly selective mu, delta or kappa antagonists. Only

the selective mu-antagonist β-funaltrexamine blocked dynorphin self-administration. It was concluded that mu receptors in the CA3 region of hippocampus are important target sites for dynorphin reinforcement.

B. Dopamine Receptor Subtypes in Behavioral Reinforcement. (Self, D.W. and Stein, L., *Soc. for Neurosci. Abstracts*, in press.)

The reinforcing properties of the selective D_2 agonists N-0437 and N-0923 were demonstrated for the first time in our cellular operant conditioning experiments. If cellular and behavioral reinforcement mechanisms are interrelated, N-0923 should also serve as an effective reinforcer of behavior. Rats were trained in daily 3-hour sessions to intravenously self-administer cocaine (0.75 mg/kg/injection) by pressing a bar. A second bar delivered no injections and provided a control for nonspecific stimulation. After cocaine self-administration had stabilized, various doses of N-0923 or d-amphetamine were substituted for the cocaine reinforcement. N-0923 was avidly self-administrated, and in fact was substantially more potent than either amphetamine or cocaine. In a second experiment, we attempted to determine the relative contribution of D_1 and D_2 receptor activation to the reinforcing action of cocaine. Cocaine self-administering rats were pretreated before the test ion with either the D_1 agonist SKF 38393 or the D_2 agonist N-0923. It is well established that if a self-administering rat is pretreated with a reinforcement enhancer (such as cocaine itself), the average interval between successive self-administrations is increased and the self-administration rate is decreased; on the other hand, if the pretreatment drug blocks reinforcement, the inter-injection interval is shortened and the self-administration rate is increased. Cocaine self-injections were decreased in a dose-dependent manner by the D_2 agonist N-0923 and increased in a dose-dependent manner by the D_1 agonist SKF 38393. The results support the idea that D_2, but not D_1, receptor activation facilitates the reinforcing action of cocaine.

Work initiated in a previous grant period on the role of endogenous opioids in reinforcement function was analyzed and published this year (Trujillo, K.A., Belluzzi, J.D., and Stein, L., *Psychopharmacology*, 104:265-274, 1991.) This work showed that very low doses of naloxone, without effect when tested by themselves, can block the reinforcing effects of amphetamine in conditioned place preference. These results provide evidence of interactions between endogenous opioids and catecholamines in the mediation of reinforcement processes.

III. Project Personnel

Dr. B. Xue has performed the technically demanding electrophysiological experiments, with great skill. David Self, a senior graduate student, has replaced Dr. E. Sehitoglu as supervisor of the behavioral work. Drs. McAfee and Belluzzi have separate support and Mr. Self is almost entirely supported by a PHS fellowship.

IV. References

Stein, L. and Belluzzi, J.D. Cellular investigations of behavioral reinforcement. *Neurosci. and Biobehavioral Reviews*, *13*:69-80, 1989.

Trujillo, K.A., Belluzzi, J.D. & Stein, L. Effects of opiate antagonists and their quaternary analogues on nucleus accumbens self-stimulation. *Behavioural Brain Research, 33*:181-188, 1989.

Trujillo, K.A., Belluzzi, J.D. & Stein, L. Opiate antagonists and self-stimulation: extinction-like response patterns suggest selective reward deficit. *Brain Research, 492*:15-28, 1989.

Trujillo, K.A., Belluzzi, J.D. & Stein, L. Naloxone suppression of self-stimulation is independent of response difficulty. *Pharmacol. Biochem. & Behav., 33*:147-155, 1989.

Self, D.W. and Stein, L. Pertussis toxin blocks morphine self-administration in the CA3 region of the hippocampus. *Soc. for Neurosci. Abstracts, 15*:35, 1989.

Grauer, E., Lutz, D.L., and Stein, L. Hippocampal self-administration of morphine and cocaine. *Soc. for Neurosci. Abstracts, 15*:35, 1989.

Stevens, K.E. and Stein, L. Mu receptors may mediate hippocampal endogenous opioid reinforcement. *Soc. for Neurosci. Abstracts, 15*:36, 1989.

Xue, B.G. and Stein, L. Opposite action of dopamine and glutamate in operant conditioning of hippocampal CA1 cells. *Soc. for Neurosci. Abstracts., 16*:261, 1990.

Truijillo, K.A., Belluzzi, J.D. and Stein, L. Naloxone blockade of amphetamine place preference conditioning. *Psychopharmacology, 104*:265-274, 1991.

Stevens, K.E., Shiotsu, G. and Stein, L. Hippocampal mu-receptors mediate opioid reinforcement in the CA3 region. *Brain Res., 545*:8-16, 1991.

Xue, B.G. and Stein, L. Reinforcement of hippocampal CA1 bursting by cannabinoid receptor activation. *Soc. for Neurosci. Abstracts.*, in press.

Self, D.W. and Stein, L. Opposite effects of dopamine D_1 and D_2 Agonist Pretreatment on cocaine self-administration. *Soc. for Neurosci. Abstracts.*, in press.

Self, D.W. and Stein, L. Receptor subtypes in opioid and stimulant reward. *Pharmacol. and Toxicology*, in press.

Larry Stein, Ph.D.
Professor and Chair